Journal of Physiology (1990), **426**, *pp.* 229–240
With 7 figures
Printed in Great Britain

LOW-THRESHOLD MECHANORECEPTIVE AND NOCICEPTIVE UNITS WITH UNMYELINATED (C) FIBRES IN THE HUMAN SUPRAORBITAL NERVE

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(*Received 9 January 1990*)

SUMMARY

1. In recordings from the human supraorbital nerve with tungsten microelectrodes, eleven afferent units with unmyelinated (C) axons were identified on the basis of their conduction velocities (0·6–1·4 m/s).

2. Eight units had low mechanical thresholds ($\leqslant$ 0·23 g) and could be activated up to their maximal firing rates of about 100 impulses/s by weak tactile stimuli, whereas three units had higher thresholds (5·5 g) and responded vigorously to noxious stimuli only.

3. During a skin indentation the low-threshold units adapted to an irregular low-frequency discharge, and release of the stimulus elicited a prominent off-response often ending with an after-discharge. Slow stroking was a particularly effective stimulus, even when done with cotton wool, whereas rapid stroking reduced the response. All types of stroking stimuli were occasionally followed by after-discharges. Repeated mechanical stimulation at short intervals resulted in a decline of the response, indicating receptor fatigue. For two units a response to skin cooling was observed.

4. The above low-threshold C units have all the main characteristics of the C mechanoreceptors known from the cat and primates but not previously proven to exist in man. The high-threshold C units are similar to the polymodal nociceptors found in other human skin areas.

INTRODUCTION

In recent years the innervation of the human face has been explored with the microneurographic technique (Vallbo & Hagbarth, 1968), in which tungsten microelectrodes are inserted percutaneously into individual nerve fascicles. Recordings have been made from afferent myelinated fibres in the infraorbital nerve (Nordin, Hagbarth, Thomander & Wallin, 1983; Johansson, Trulson, Olsson & Abbs, 1988*a*; Johansson, Trulson, Olsson & Westberg, 1988*b*; Nordin & Hagbarth, 1989; Nordin & Thomander, 1989), from sympathetic C fibres in the supraorbital nerve (Nordin, 1990) and from motor fibres in the facial nerve trunk (Nordin, Hagbarth, Thomander & Wallin, 1986). The present study on afferent C fibres in the supraorbital nerve provides the first proof of the presence of low-threshold C mechanoreceptors in humans and also establishes the existence of mechano-heat nociceptors in the forehead.

METHODS

Subjects

Eleven afferent C units were recorded in five experiments performed on the supraorbital nerve of three healthy male subjects aged 22–23 years. These successful attempts were part of a series of more than sixty experiments (in thirty-one subjects), in which recordings from other nerve fibre types were obtained. The subjects gave their informed consent, and the study was approved by the local ethics committee.

Nerve recordings

The methods used were similar to those described for recording of afferent C units in skin nerves of the extremities (Torebjörk, 1974; Torebjörk & Hallin, 1974). A lacquer-insulated tungsten microelectrode, with a shaft diameter of 0·2 mm and a tip diameter of about 5 μm, was inserted through the skin 1–15 mm above the eyebrow, and a reference electrode was inserted nearby. The search for an intrafascicular recording position was aided by the subject's reports of cutaneous paresthesiae evoked by electrical stimulation via the microelectrode. The signal was amplified and bandpass filtered (usually 500–5000 Hz), and the signal-to-noise ratio was improved by an amplitude discriminator which eliminated the central portion of the noise. The neurogram was audio-monitored. In more detailed studies of potential waveforms (as in Fig. 1), noise reduction was not used and the low-cut filter was at 200 Hz.

For the single units recorded for the longest periods the conduction velocity was determined by electrical stimulation via two needle electrodes, which were inserted into the skin of the receptive zone. Since this involved a substantial risk of losing the recording position because of facial movements, the conduction velocities of the other units were estimated instead from the responses to rapid tap stimuli delivered with a blunt probe attached to a force transducer (peak force 0·1–0·5 N). The shortest well-defined latency was used in the calculations. The surface distance from the stimulation point to the insertion point of the microelectrode was measured, and correction of the conduction distance for the position of the electrode tip was made by trigonometric calculations.

Testing procedure

Receptor characteristics were tested by manually applied stimuli. A set of calibrated von Frey filaments (51, 23, 10, 5·5, 1·3, 0·6, 0·23, 0·09 and 0·06 g) was used for threshold determinations and to study the response to skin indentation. Mechanical stimuli were also delivered with a smooth plastic probe (tip diameter about 1 mm) attached to a strain-gauge force transducer, and with cotton wool. The noxious stimuli used were scratches with an injection needle and application of a glowing match, each of which could be attached to the force transducer. Local skin cooling was produced by evaporation of a drop of ether or, in one case, by spraying for a few seconds with ethyl chloride. For some units, the skin of the receptive field was stretched, or groups of hairs arising from the receptive field were moved with a probe without touching the skin.

Signal display and analysis

During the experiments the nerve and force signals were monitored on a storage oscilloscope or a video display monitor. They were stored on tape (FM tape-recorder, Sangamo Sabre VI, USA) and later recorded on paper (ES1000, Gould Instruments, France). A universal waveform analyser (Data 6000A-611, Data Precision, USA) connected to a plotter was used for studying potential waveforms and measuring latencies and interspike intervals. The instantaneous firing rates of the units were displayed in dot diagrams by a computer program.

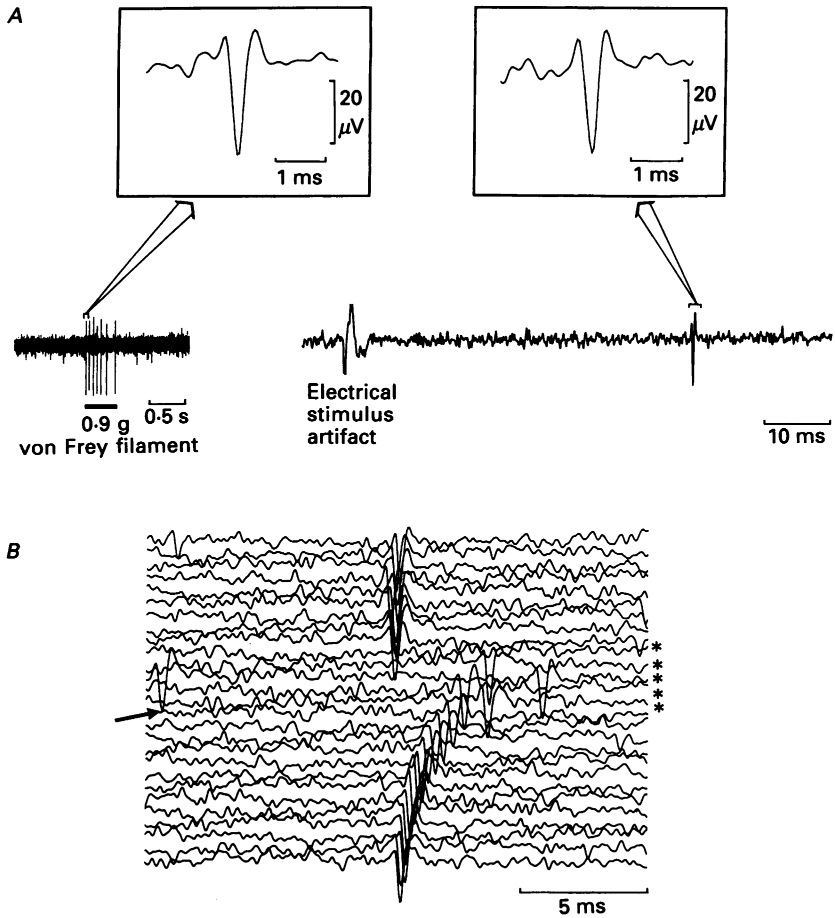

Fig. 1. Recordings from a low-threshold C unit in the human supraorbital nerve. *A*, response to a von Frey filament of 0·09 g, and to electrical intradermal stimulation. Note (above) that the mechanically and electrically evoked nerve action potentials, superimposed on the background noise, were similar. The latency of the response to electrical stimulation was about 53 ms, which corresponded to a conduction velocity of 1·3 m/s. *B*, this latency was increased by simultaneous mechanical activation, further indicating that the same unit was responding. Twenty-seven consecutive responses to electrical stimuli delivered at a rate of 0·9/s are shown from above to below. The initial 44 ms of the post-stimulus period have been omitted. The asterisks indicate a period of pressure in the receptive field. The arrow points to one of the mechanically evoked discharges.

RESULTS

Low-threshold mechanoreceptive C units

Recordings were obtained from eight afferent C units which responded to weak tactile stimuli (Fig. 1*A*). The von Frey thresholds were 0·23 g for four units and 0·09 g for another two. The remaining two units responded to 0·06 g, but their thresholds might have been even lower, since weaker stimuli were not used.

For two units the conduction velocities were determined by electrical stimulation, and were 1·3 and 0·6 m/s (Figs 1*A* and 2). As illustrated, the mechanically and electrically evoked potentials were similar (Fig. 1*A*, above), and simultaneous mechanical stimulation caused an increase in the latency of the electrically evoked

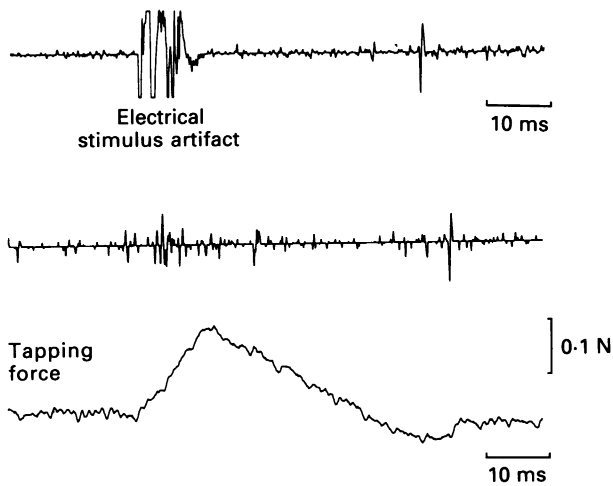

Fig. 2. Comparison of the response latency for a low-threshold C unit following electrical intradermal stimulation (above) and a rapid tap stimulus with a probe attached to a force transducer (below). On electrical stimulation the response latency was 43 ms, which corresponded to a conduction velocity of 0·6 m/s. In the trial illustrated, the latency of the tap-stimulus response was 5 ms longer, and on average this difference was about 7 ms. Note the low-amplitude response from rapidly conducting myelinated fibres immediately after the onset of the tap stimulus. Bandpass filtering and noise reduction have distorted the potential waveforms.

response (Fig. 1*B*, only studied for this unit), indicating that the discharges originated from the same axon. For this and all other C units in the present study the recorded action potential was triphasic, with a duration of 1–1·5 ms, and the major deflection was in the negative direction.

The conduction velocities of the remaining six units were estimated from the responses to tap stimuli. For the above two units the latency of the tap-stimulus response exceeded that of the electrically evoked response by somewhat less than 10 ms (cf. Fig. 2), and allowance for this difference was therefore made by subtracting 10 ms. When approximated in this way, the conduction velocities were 0·7–1·4 m/s.

The receptive fields of the units, determined by means of a suprathreshold von Frey filament (5·5 g), are depicted in Fig. 3 (open areas). The field sizes were 34–155 mm^2 (85 ± 4 mm^2, mean ± S.D.). Within the fields there was some variation in sensitivity, but this was not studied in detail.

All eight units responded in a similar way to a clearly suprathreshold, sustained skin indentation (Fig. 4*A* and *B*). Generally, the initial peak firing rates were 60–90 impulses/s, although spike intervals corresponding to 90–110 impulses/s were occasionally observed. There was marked adaptation during the first second, and the following plateau response was sluggish and irregular. The average firing rates during the first five 1 s periods of the response were 41, 17, 14, 12 and 12 impulses/s, when calculated from the trial with the most sustained discharge for each unit. The adaptation varied somewhat between different points within a receptive field and was sometimes complete (cf. Fig. 4*B*).

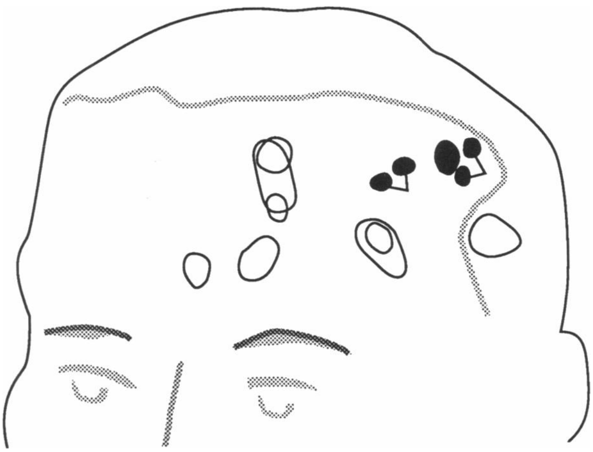

Fig. 3. Receptive fields of eleven afferent C units sampled from the supraorbital nerve. The open fields, mapped with a von Frey filament of 5·5 g, correspond to the low-threshold units. The filled fields, mapped with a 23 g filament, correspond to the high-threshold units. The angles indicate that two of the latter units had double receptive fields separated by an unresponsive zone (this was ascertained by comparing the potential shapes evoked from the two fields and checking that they were similar and changed in parallel during minor adjustments of the microelectrode position). Three fields obtained on the right-hand side are depicted in the corresponding left area.

The termination of a skin indentation resulted in a prominent off-response, even if the static discharge had ceased. Although the stimulator was retracted rapidly, this response often lasted 0·5–1 s, forming an after-discharge (Fig. 4*A* and *B*). Such prolonged firing was most pronounced for the strongest stimuli. In all except one unit, examples of after-discharges following the response to a rapid tap stimulus were observed (Fig. 4*C*). During an after-discharge there was a gradual decrease in firing rate (most clearly seen in Fig. 4*C*).

For all units gentle stroking in the receptive field was a particularly effective stimulus. When this was done with cotton wool, peak firing rates of 60–100 impulses/s regularly occurred (Fig. 5*A*), and higher frequencies were not observed even in response to scratching with a needle (Fig. 5*B*). All types of stroking stimuli were occasionally followed by after-discharges (e.g. Fig. 5*A*). These had a tendency to last somewhat longer than the after-discharges following release of skin indentation, and the maximal duration observed was 11 s.

The effects of stroking at different velocities with a blunt probe were studied for three units. As exemplified in Fig. 5*C*, slow probe movements (about 5–10 mm/s) were more effective than faster ones (about 50–100 mm/s) and yielded both higher peak firing rates and a larger total number of spikes in the response. This was the case even when greater force was applied during rapid stroking.

As seen in Fig. 5*D*, intensive stroking with a probe in a part of the receptive field caused a reduction of the response, almost to the point of inexcitability. This fatigue

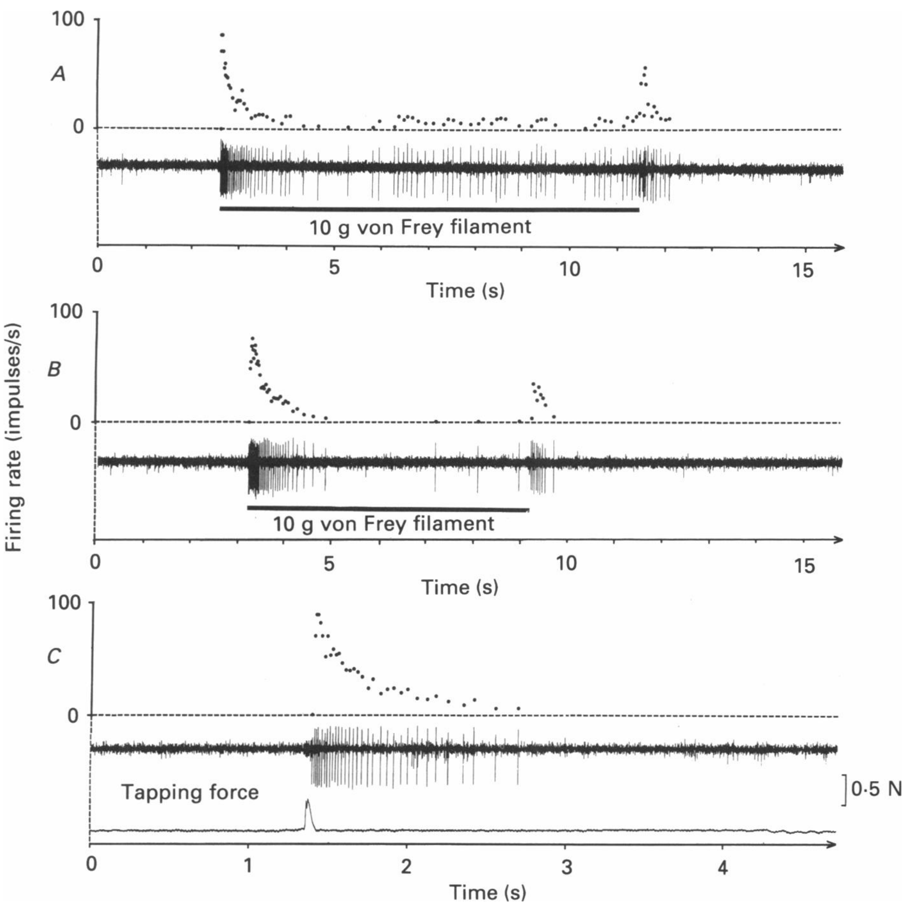

Fig. 4. Typical responses of the low-threshold C units to skin indentation. *A* and *B*, trials with a von Frey filament of 10 g in different parts of a receptive field. Note the marked initial adaptation, the irregular low-frequency discharge or almost complete adaptation during sustained indentation, and the off-response terminating with an after-discharge. *C*, rapid tap stimulus evoking a response followed by an after-discharge.

was restricted to the stimulated part of the field, and the sensitivity recovered after a period without stimulation. For six other units studied with repeated skin indentations at short intervals (< 15–20 s), fatigue of the response was also observed.

Skin cooling with ether evoked a response as shown in Fig. 6*A*. For this unit the firing rates during the initial part of the evaporation phase were 15–25 impulses/s, and the discharge lasted for up to 4 s. Similar cooling responses were observed for another unit tested with ethyl chloride. For these units noxious heat did not evoke any activity that could not be explained by the simultaneous mechanical stimulation. The same two units were sensitive to skin stretching, which gave rise to dynamic

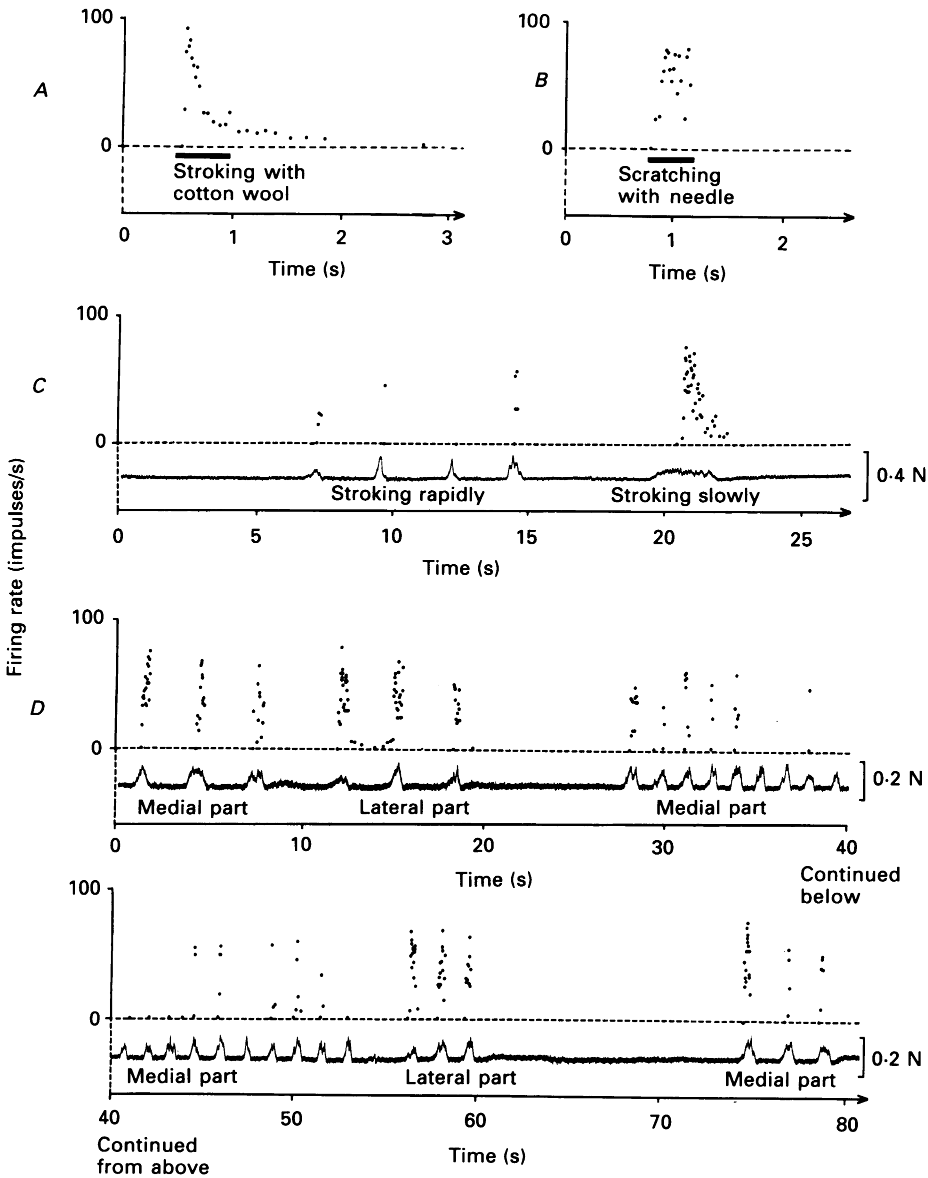

Fig. 5. Characteristic firing patterns of the low-threshold C units in response to stroking stimuli. *A*, stroking with cotton wool resulted in a vigorous response, in this trial followed by an after-discharge. *B*, for the same unit scratching with a needle did not yield higher firing rates. *C*, effects of stroking at different velocities with a blunt probe attached to a force transducer. Note that a slow stroking movement (right), in spite of the lower force applied, was a much more effective stimulus than faster ones. *D*, signs of local receptor fatigue induced by a period of intensive stroking with a blunt probe in the medial part of a receptive field. The response evoked from the lateral part was unaffected. Note the recovery of the sensitivity after a period without stimulation. In the groups of three stroking stimuli there were also signs of fatigue with a successive reduction of both the firing rates and number of spikes. This was most pronounced for the last group of three trials.

responses, and strong stretching also resulted in a weak static discharge (Fig. 6*B*). In the absence of any intentional stimulation, a low degree of resting activity (< 2 impulses/s) was observed for one of them, and for another unit. The unit with scalp hairs arising from its receptive field responded vigorously to hair movements, but

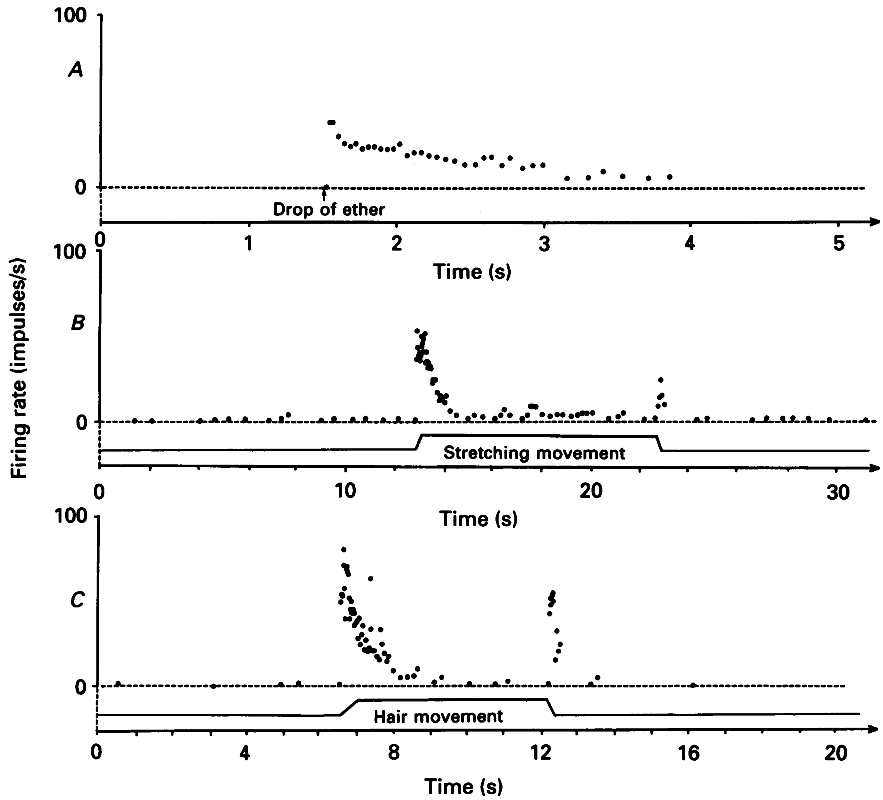

Fig. 6. *A*, response of a low-threshold C unit to skin cooling induced by evaporation of a drop of ether. In a control trial (not illustrated) a drop of water at room temperature, which was a stronger and more lasting mechanical stimulus, caused only a weak discharge of the unit, indicating that the response to ether was really an effect of cooling. *B*, response of another low-threshold C unit to stretching of the skin of the receptive field. The time course of the stimulus is indicated schematically. *C*, the same unit as in *B* responded to movement of a group of hairs. There was no response to sustained displacement of hairs other than that explicable by inadvertent movements. The time course of the stimulus is indicated schematically.

there was little or no response to sustained displacement of hairs (Fig. 6*C*). This unit could be activated even by movement of single hairs.

Nociceptive C units

Three units with conduction velocities of 0·8–1·3 m/s (determined by electrical stimulation) were easily distinguished from the above units, since they had higher threshold (5·5 g) and responded vigorously to noxious stimuli only. Their receptive fields, mapped with a 23 g von Frey filament, are shown in Fig. 3 (filled areas). As

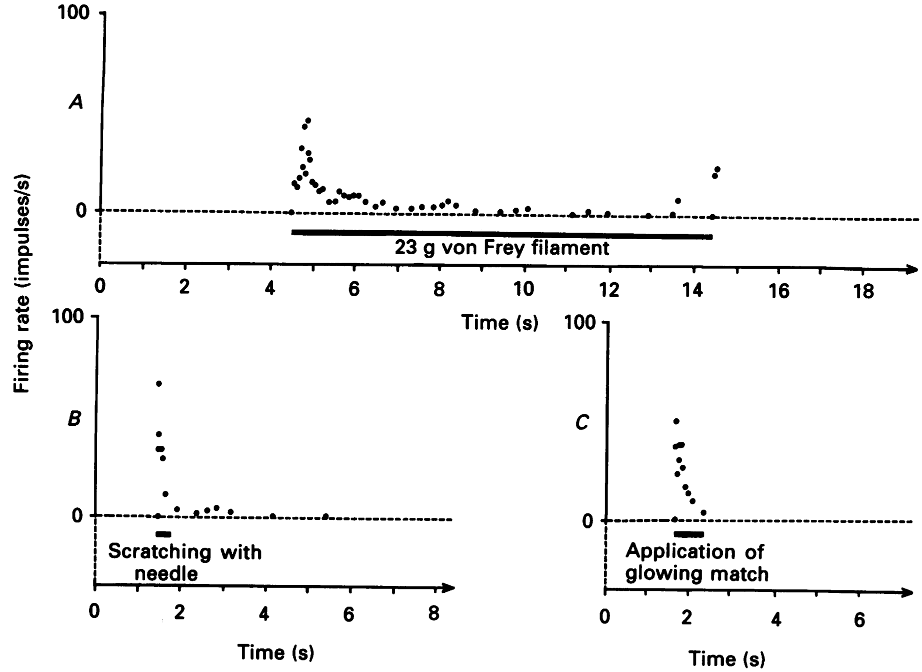

Fig. 7. Typical responses of the high-threshold C units. *A*, skin indentation with a von Frey filament of 23 g. *B*, scratching with a needle; note the after-discharge. *C*, application of a glowing match, resulting in a response without after-discharge. In a control trial (not illustrated) skin indentation alone with the same force evoked no response.

indicated, two of them had double fields separated by an unresponsive zone. The total field sizes were 46–61 mm^2.

The response of these units to a clearly suprathreshold skin indentation (23 or 51 g) was different from that of the low-threshold units (Fig. 7*A*). Hence, the peak firing rate was lower (25–60 impulses/s), the off-response was less pronounced or absent, and there was no after-discharge. In the trials with the most pronounced static discharge, the average firing rates during the first five 1 s periods were 19, 6, 5, 3 and 3 impulses/s.

Scratching with a needle evoked a response with maximal firing rates of 40–70 impulses/s, followed regularly by an after-discharge lasting up to 5 s (Fig. 7*B*). Noxious heat was also an effective stimulus (Fig. 7*C*), and firing rates of about 100 impulses/s were observed in some trials. There were no after-discharges in response to heat stimuli, however. Skin cooling evoked no response when tested initially, but for one unit a short discharge occurred when the test was repeated after a series of trials with heat. For two units evidence of increased mechanical sensitivity subsequent to heat stimulation was observed.

DISCUSSION

The high-threshold C units in the present study have properties similar to those of the polymodal nociceptors recorded in the skin nerves of the extremities, including signs of sensitization by noxious heat (Torebjörk, 1974; Torebjörk, LaMotte &

Robinson, 1984). The low-threshold C units, on the other hand, are of a type which has not previously been identified with certainty in man. However, they have all the main characteristics of the C mechanoreceptors studied in the cat (Zotterman, 1939; Douglas & Ritchie, 1957; Iggo, 1960; Iggo & Kornhuber, 1968; Bessou & Perl, 1969; Bessou, Burgess, Perl & Taylor, 1971; Hahn, 1971; Iggo & Kornhuber, 1977) and in primates (Kumazawa & Perl, 1977). These include their firing pattern during skin indentation with 'intermediate' adaptation and a pronounced off-response, a tendency to give after-discharges, preferential sensitivity to slowly moving mechanical stimuli, fatigue caused by repeated stimulation, and excitation by skin cooling. It has not been possible to identify such C mechanoreceptors in microneurographic recordings from the human radial, peroneal or saphenous nerves, in spite of searches for them in experiments devoted to the study of afferent C units (Torebjörk, 1974; Torebjörk & Hallin, 1974). One reason for this may be that the latter recordings were obtained from nerves innervating distal skin areas, since, in primates, the C mechanoreceptors are more rare distally (Kumazawa & Perl, 1977). Furthermore, it may be presumed that the C mechanoreceptors, being 'primitive' sense organs, are comparatively rare in man. At least they are less common in primates than in the cat (Kumazawa & Perl, 1977). In their recordings from the human infraorbital nerve, Johansson *et al.* (1988*b*) found *one* low-threshold mechanoreceptive afferent which they suggested was unmyelinated on the basis of its long-latency response to skin indentation (no electrical stimulation was performed). From the limited data given it seems probable that this was the same type of unit as described in the present report.

The relatively short mechano-electrical transduction time (< 10 ms) indicated by the present study may seem incompatible with the 'minimal contact time' of 40–80 ms reported for C mechanoreceptors in the cat (Bessou *et al.* 1971). However, the latter figures were obtained for stimuli 4–6 times higher than the threshold, whereas the tap stimuli used in the present study were at least 40 times the threshold. The above authors also noted that the duration tended to decrease with stronger stimuli.

In his original description of C mechanoreceptors in the cat, Zotterman (1939) suggested that they are of importance for tickling sensations. The present data are consistent with this view, since the human C mechanoreceptors responded vigorously to gentle stroking on the forehead, a well-known and effective tickling stimulus. Furthermore, the observed after-discharges might explain why the sensation often outlasts the stimulation (cf. Zotterman, 1939), and the receptor fatigue may account for the abolition, by previous scratching of the skin, of the tickling sensation in response to an adequate stimulus (cf. Zotterman, 1972). However, the results of pressure blocks indicate that a tickle on the dorsum of the hand is mediated by myelinated fibres, and not by C fibres (Hallin & Torebjörk, 1976); it is conceivable that sensations described as tickling may arise from activation of various types of mechanoreceptors, and that there are regional differences.

In theories concerning the pathogenesis of trigeminal neuralgia, the existence of C mechanoreceptors in the face must be taken into consideration. In fact, most of the characteristics of the stimuli triggering the pain may be accounted for by C mechanoreceptors. These include the greater effect of stroking stimuli than firm pressure, and the precipitation of attacks by sudden release of pressure, hair movements and facial contractions (cf. Kugelberg & Lindblom, 1959). On the other hand, the enhancement of the triggering effect by an increase in vibration frequency (Kugelberg & Lindblom, 1959) does not fit with the properties of C mechanoreceptors in the cat (Bessou *et al.* 1971).

This work was supported by the Swedish Medical Research Council and the 'Förenade Liv' Mutual Group Life Insurance Company, Stockholm, Sweden. The author is indebted to Professors Karl-Erik Hagbarth and Erik Torebjörk for valuable comments on the manuscript, and to the technician Catarina Färnstrand for help in preparing the figures.

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